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Limacella bangladeshana, first record of the genus in Thailand

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Abstract—Mushroom specimens collected in Thailand were identified as *Limacella bangladeshana*, based on morphology and LSU and ITS sequence analyses. A full description and illustration are provided. This is the first record of the genus *Limacella* from Thailand.

Key words—*Agaricales*, *Amanitaceae*, gill fungus, taxonomy

Introduction

Limacella (*Amanitaceae*, *Agaricales*) was described by Earle (1909) with *L. delicata* (Fr.) Earle ex Konrad & Maubl. as the type species. This genus is mainly distributed in Europe and America, but some species are found in Asia (Smith 1945; Hongo 1978; Pegler 1983, 1986; Yang & Chou 2002; Neville & Poumarat 2004; Sato & al 2010; Ferreira & al. 2013; Kumari & al. 2013; Yang 2015; Hosen & Li 2017; Nascimento & Wartchow 2018; Yang & al. 2018). Approximately 47 taxa of *Limacella* are known (http://www.indexfungorum), but no *Limacella* species have been reported from Thailand (Chandrasrikul & al. 2011). During a taxonomic survey of macrofungi collected in northern Thailand, we found specimens that corresponded to the description of *L. bangladeshana*, previously reported from Bangladesh (Hosen & Li 2017). Herein we describe and illustrate the morphological characteristics of the Thai material and provide supporting evidence based on LSU and ITS gene sequence matches in GenBank.

Materials & methods

Morphology

Basidiomata were collected from the campus of Chiang Mai University, Chiang Mai Province, Thailand, and kept in plastic collection boxes until transported back to the laboratory. Macroscopic descriptions are based on observations of the fresh basidiomata. Color names and codes follow Kornerup & Wanscher (1978). The specimens were dried at 40–45 °C in an electric food dryer. The micromorphological data were derived from dried specimens mounted in 95% ethanol and rehydrated in distilled water, 3% KOH, or Melzer's reagent. Dimensions of anatomical features were calculated from at least 50 measurements of each structure. For basidiospores, Q represents the range of length/width ratios from individual spores and is the mean ± standard deviation of the ratios from all measured spores. Dried specimens were deposited in the herbarium of Research Laboratory for Excellence in Sustainable Development of the Biological Resources, Faculty of Science, Chiang Mai University, Thailand (SDBR-CMU).

Sequence analyses

Genomic DNA was extracted from fresh specimens using a Favorgen DNA Extraction Mini Kit following the manufacturer's instructions. The large subunit (LSU) region of ribosomal DNA (rDNA) was amplified by polymerase chain reaction (PCR) with LROR/LR5 primers, and the rDNA internal transcribed spacer (ITS) region was amplified with ITS4/ITS1F primers, under the following thermal conditions: 94 °C for 2 min; 35 cycles of 95 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min; and 72 °C for 10 min. PCR products were checked under UV light on 1% agarose gels stained with ethidium bromide and purified using a NucleoSpin Gel and PCR Clean-up Kit following the Macherey-Nagel protocol. The purified PCR products were directly sequenced according to the Sanger method by 1ST Base Company in Kembangan, Malaysia, using the same PCR primers cited above. Sequences were used to query GenBank database via BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Taxonomy

Limacella bangladeshana Hosen, Phytotaxa 332: 281 (2017). Fig. 1

Pileus 20–50 mm diam., subconical to sub-hemispherical, becoming planoconvex, surface dry, orange white (5A2) to brown (7E4), slightly darker at the centre; margin non-striate, with brownish orange (6C4) to light orange (5A4) at edge, covered with white velvety to cottony squamules. Context 5–8 mm thick at the center of the pileus, white, unchanging when cut or bruised. Lamellae 22–30 × 7–10 mm, free, white to brownish orange (6C4) or brown (7E4) to dark brown (7F4) when dried. Stipe 25–50 × 5–12 mm, cylindrical to subcylindrical, slight swollen at base, dry, not slimy,

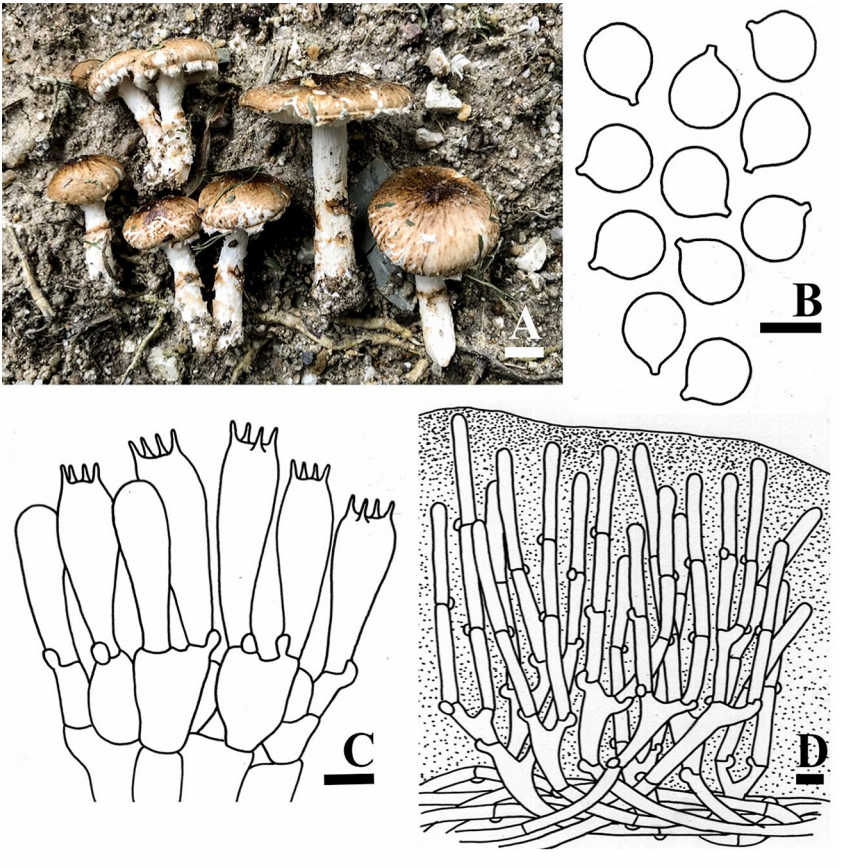

Fig. 1. *Limacella bangladeshana* (SDBR-CMU-JK0146).
A. basidiomata; B. basidiospores; C. basidia; D. pileipellis.
Scale bars: A = 10 mm; B, C = 5 µm; D = 10 µm.

surface covered with fibrillose or floccose squamules, white to brownish orange (6C4). Annulus absent.

Basidiospores 3–5 × 3–4.5 µm, Q = 1.00–1.11, = 1.04 ± 0.06, globose to (rarely) subglobose, hyaline, inamyloid, thin-walled, smooth; apiculus cylindrical to subcylindrical ≤1.2 µm long. Basidia 20–30 × 5–8 µm, clavate, hyaline, thin-walled, 4-spored, with sterigmata ≤4 µm long, with basal clamp connections. Subhymenium ≤35 µm thick, with 2–3 layers of inflated to subglobose cells, thin-walled, hyaline. Hymenophore trama

bilateral, filamentous hyphae 3–9 µm diam. mixed with inflated cells 15–32 × 10–20 µm. Lamellar edge fertile. Cheilocystidia and pleurocystidia absent. Pileipellis an ixo-trichodermium composed of more or less erect hyphae embedded in a gelatinized matrix, hyphae septate, hyaline, cylindrical, branched, with terminal cell 25–75 × 5–8 µm, clamp connections present. Stipe surface hyphae interwoven, filamentous, hyaline, thin-walled 3–5 µm diam., cells not inflated; stipe trama hyphae longitudinally arranged, 4–8 µm diam., mixed with acrophysalidic cells 10–16 µm diam. thin-walled, hyaline to pale yellow, clamp connections rare.

Specimens examined—**THAILAND, Chiang Mai Province, Muang District,** Chiang Mai University, 18°48′03″N 98°57′21″E, elevation 334 m, on soil, 2 June 2018, J. Kumla & N. Suwannarach (SDBR-CMU-NK0356; GenBank MK288133, MK290392); 3 June 2018, J. Kumla (SDBR-CMU-JK0146, GenBank MK290393, MK290394).

Molecular analysis

The LSU and ITS sequences of specimens SDBR-CMU-NK0357 and SDBR-CMU-JK0146 were deposited in GenBank. Our LSU and ITS sequences were 100% similar to *L. bangladeshana* ex-type sequences KR816866 and KR816865.

Discussion

The agaricoid basidiomata, slightly glutinous pileus, a fertile lamellar edge, lack of annulus, a stipe that is not viscid, and a trichodermium pileipellis embedded in a gelatinous matrix support the placement of our collections in *Limacella* (Singer 1986, Tulloss & al. 2016). According to the descriptions provided by Singer (1986), this genus is divided into two sections based mainly on the stipe characters. There are *L.* sect. *Lubricae* (viscid or glutinous stipe) and *L.* sect. *Limacella* (dry stipe). Based on its dry stipe, *L. bangladeshana* belongs to *L.* sect. *Limacella* (Hosen & Li 2017).

Thirteen *Limacella* species have been reported from Asia: *L. anomologa* (Berk. & Broome) Pegler, *L. asperulospora* Corner, *L. bangladeshana*, *L. glioderma* (Fr.) Maire, *L. illinita* (Fr.) Maire, *L. magna* B. Kumari & R.C. Upadhyay, *L. myxodictyon* (Berk. & Broome) Pegler, *L. ochraceolutea* P.D. Orton, *L. olivaceobrunnea* Hongo, *L. quilonensis* Sathe & J.T. Daniel, *L. singaporeana* Corner, *L. subglischra* (S. Imai) S. Ito, and *L. taiwanensis* Zhu L. Yang & W.N. Chou (Corner 1994, Hosen & Li 2017, Imai 1938, Kumari & al. 2013, Pegler 1986, Sathe & Daniel 1981, Sato & al. 2010, Yang 2015, Yang & Chou 2002). *Limacella asperulospora*, *L. glioderma, L. illinita*, *L. myxodictyon*,

L. ochraceolutea, *L. olivaceobrunnea*, and *L. singaporeana* with buff white, dark cinnamon to reddish brown, pale brown, dark reddish brown, dark yellowish brown colors and larger (3.5–12.5 × 3–7 µm) basidiospores with higher (1.05–1.2) values (Hongo 1978, Pegler 1986, Corner 1994, Yang 2015) are distinguished from *L. bangladeshana*, with orange white to brown basidiomata and globose basidiospores (3–5 × 3–4.5 µm) with a lower value (1.04) (Hosen & Li 2017). The yellowish to pale orange basidiomata distinguish *L. subglischra* (Imai 1938) from *L. bangladeshana.* Shorter basidiospores separate *L. bangladeshana* from *L. anomologa* (4–5.7 × 2.7–3.3 µm), *L. magna* (7–12.5 × 5–7 µm), and *L. quilonensis* (7–10 × 3–4 µm) (Sathe & Daniel 1981, Pegler 1986, Kumari & al. 2013), while the subglobose basidiospores of *L. taiwanensis* (3–4.5 × 3–3.5 µm; Yang & Chou 2002) with a verruculose surface clearly distinguish that species from *L. bangladeshana*. The LSU molecular analysis supports the morphological differences between *L. bangladeshana* and other *Limacella* species (Hosen & Li 2017).

The LSU and ITS sequence matches in GenBank confirmed that the two Thai specimens belong to *L. bangladeshana*. Therefore, the combination of morphological and molecular characteristics supports the identification of *L. bangladeshana*, a new record in Thailand.

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